CARDIOVASCULAR, PHARMACOLOGY, CHEMISTRY

#530

Cf. #307
7/1/61 - 7/1/65

Cf. #221
1/1/59 - 7/1/60

Cf. #137
11/15/56 - 11/15/5

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Cattell, Chm.
Dr. Bing
Dr. Jacobson

Application For Research Grant

Date: January 27, 1966

1. Name of Investigator: SAMUEL BELLET, M.D.

2. Title: Director, Division of Cardiology

3. Institution & Address: PHILADELPHIA GENERAL HOSPITAL
34th Street and Curie Avenue
Philadelphia, Pennsylvania 19104

4. Project or Subject: THE EFFECT OF CAFFEINE ON BLOOD LIPIDS
   1. The Effect of Caffeine on Diabetics
   2. The Effect of Caffeine on Urinary Catecholamines
   3. The Effect of Caffeine & Smoking on Free Fatty Acids
   4. The Effect of Caffeine on Blood Coagulation
   5. Studies in the Experimental Animal
   6. Effect of Chronic Administration of Caffeine on the Lipid Fractions in Chronic Experiments
   7. Basic Levels of Lipids in Chronic Caffeine Subjects
   8. The Effect of Caffeine on the Normal Subject

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

SEE CONTINUATION SHEETS (#1 - 3)

6. Budget Plan:

a.	Salaries	$23,445.05
b.	Expendable Supplies	5,430.00
c.	Other Expenses	
d.	Permanent Equipment	4,331.26
e.	Overhead (15% of a, b, c)	
	Total	$33,206.31

7. Anticipated Duration of Work: THREE YEARS

8. Facilities and Staff Available:

SEE CONTINUATION SHEETS #5 - 6)

9. Additional Requirements:

NONE

10. Additional Information (Including relation of work to other projects and other sources of support):

NONE

Signature

Director of Project

Business Officer of the Institution: Benjamin F. Burgess, Jr.
Director of Research

1005101294

5. DETAILED PLAN OF PROCEDURE:

1. Current Status of Work in Area of this Proposal: There is very little literature relative to the relationship between caffeine and its effects on blood lipids. There is also little data concerning the relationship of caffeine administration to the prevalence of atherosclerosis. The only experimental data we were able to find was that of Czochra-Lysanowicz and Mjasknikow where caffeine was observed to produce atherosclerosis in rabbits (6, 13).

Several epidemiologic studies have recently been performed suggesting a relationship between the effect of caffeinated beverages and coronary artery disease (14, 20).

Recently, work in our laboratory has shown that both caffeine and coffee significantly elevated the free fatty acid level (4). Moreover, it is fairly well recognized that a repeated increase in plasma free fatty acids may predispose to a rise in lipoprotein lipids (17). The administration of caffeine in certain respects bears a striking parallel to that of nicotine. The effects of nicotine and smoking have been studied fairly extensively in our laboratory (2, 3, 11, 12); the effects of caffeine on FFA (0.5 Gm) are quite similar to and somewhat more prolonged than that of smoking (2 cigarettes). Smoking in humans and nicotine in dogs caused a rapid and consistent rise in serum FFA levels and patients with myocardial infarction developed an elevation more than twice that of normal subjects and non-coronary patients. In dogs given nicotine daily for a 6-week period, there was a 50% rise in the level of serum cholesterol.

Some lipolytic effects of caffeine have been studied in vivo and in vitro, many of which appear to be opposite to those of insulin (7, 18). Like epinephrine (16, 19), caffeine potentiates cyclic 3'5' AMP and phosphorylase activity (5, 9, 10, 15) and produces both glycogenolysis (5, 8) and lipolysis (4, 7, 18).

2. Specific Aims: The specific aims are to study the relationship between caffeine and blood lipids in the human subject and in the experimental animal in the acute stage and following chronic administration. Specifically, the following will be studied: (a) The effect of caffeine in normal subjects (amplification of our previously published work). This will include adults in the age group between 17-25 and those between the ages of 40-65. (b) Studies will be performed in children, ages 4-16. There are some suggestions that children may handle caffeine somewhat differently from adults. (c) The effect of caffeine in diabetics. This would seem to be of some importance since caffeine manifests an influence in the carbohydrate metabolism and caffeine and insulin have been shown to be antagonists ( ). In addition, many diabetics are coffee drinkers. (d) The simultaneous effects of caffeine and smoking. Preliminary experiments have shown that caffeine modifies the effect of smoking in blood lipids. (e) The effects of concomitant administration of caffeine and glucose. (f) The effects of beta-blocking agents on the administration of caffeine in order to determine whether the FFA production is abolished in a manner similar to that of epinephrine and nicotine. (g) The effects on blood coagulation in the human subject (see below). (h) The study of the basic level of triglycerides and cholesterol in human subjects who have been drinking coffee for years.

3. Methods of Procedure: Twenty-five normal subjects, aged 17-25, and 25 subjects, aged 40-65, will be studied in the fasting state. The subjects will be kept at rest and free of environmental stimulation and will be prohibited from smoking during the experiment. The following series of observations will be made on these groups. On one day, 5 Gm of soluble instant coffee powder will be ingested over a 15-minute period. In a second series of experiments, the patients will be given 500 cc. of hot water with 3 tablets of sweetener as a control. In the third series, caffeine sodium benzoate (0.5 Gm of each buffered in saline) will be given intramuscularly. Blood samples taken from an atencubital vein will be taken before, 30,60,90,120 and 180 minutes after the ingestion of the beverage. Since our previous experiments have shown that the effects on FFA extends over 180 minutes, we will extend our period of administration(on some patients) after caffeine administration to 4 hours. Serum FFA concentrations will be determined in all experiments. Serum triglycerides, cholesterol and plasma glucose will also be determined.

Similar studies will be performed in children who will be given caffeine in doses of 8-10 mg/Kg.

Studies similar to the normal group will be repeated in patients with coronary artery disease; included will be subjects with previous myocardial infarction. Studies of this group should be of interest since it has been shown that these subjects have increased sympathetic tone and show an increase in circulating catecholamines with higher control levels of FFA. Statistical significance of the data obtained will be determined by means of the t-test.

Studies of Diabetics: Patients will come to the Hospital at 8:00 a.m., having had nothing to eat or drink since midnight and no insulin for 24 hours. Blood will be drawn for blood sugar and plasma FFA. 500 mg of caffeine sodium benzoate will be administered parenterally. Blood will be drawn for blood glucose and plasma FFA at 30, 60,90,120 and 180 minutes after the administration of caffeine sodium benzoate. Subsequently, the same patients will be studied in a similar manner but without receiving the caffeine sodium benzoate.

Studies on Urinary Catecholamines: Ten experiments will be performed in which the urine will be studied for catecholamines during a control period of 4--6 hours and for a similar period after caffeine has been administered. The purpose of this study is to determine the effect of caffeine administration on urinary catecholamines and to compare these values with those of other preparations that produce an increase in FFA, e.g., epinephrine and nicotine (9, 10, 12).

Effect of Caffeine and Smoking on Free Fatty Acids: This will be determined in subjects where the effects of caffeine and nicotine on separate days have been observed and the effects on FFA noted. The combination of these two will then be studied. Preliminary studies have indicated that the rise in FFA, instead of being additive, is much less marked.

Blood Coagulation Studies: We propose to study the coagulation profile of caffeine in the human subject and in the experimental animal after the administration of 2½ cups of coffee (containing 218 mg of caffeine) and after 500 mg of caffeine. The following clotting studies will be performed. (1) The clotting time in glass and silicone; (2) Plasma studies consisting of (a) One-stage prothrombin time of plasma, (b) Stypzen time, (c) Plasma fibrinogen concentrations, (d) Recalcification time of the plasma, (e) Heparin tolerance times, and (f) Thromboelastography; and (3) Fibrinolytic studies consisting of (a) Euglobulin lysis times, (b) Zones of lysis of fibrin plates, and (c) Urokinase lysis times.

Studies in the Experimental Animal: The following series of acute experiments will be performed on dogs involving the administration of caffeine in a dose of 20-25 mg/Kg body weight. Control samples will be taken before and at intervals of ½ hour to 4 hours. These will be studied for FFA. Preliminary experiments have shown a uniform rise of FFA with these experiments. The effects of methoxamine, hexamethonum, reserpine, Inderal, C-60 will be studied. The purpose will be to determine the degree of blocking effects on FFA production by the various drugs administered.

The Effect of Chronic Administration of Caffeine on the Lipid Fractions in Chronic Experiments: At the present time we have been studying a series of dogs in whom caffeine has been administered daily for a period of 6 weeks. It is too early to determine significant effects. We plan to utilize the rabbit, rat and squirrel monkey for such chronic experiments. We feel that the result should be of some interest.

Basic Levels of Lipids in Chronic Caffeine Subjects: It is proposed to determine the levels of triglycerides and cholesterol in a group of 200 subjects between the ages of 40-65 who have been drinking 4-6 cups of coffee/day over a period of years. This data will be compared to a similar group of non-coffee drinkers (or those who drink only 1-2 cups/day).

3. Significance of this Research: As atated above, the possible connection between caffeine and coronary heart disease is of primary importance. Keys to the mechanism of FFA response and lipoprotein-lipid increments are important steps in this direction. Because coffee is one of the most widely consumed beverages in the United States, data relating to the physiologic effects of caffeine is highly significant. In general, these findings will be of interest in view of the suggested relationship of FFA elevation to increments in other lipid fractions, notably, triglycerides and cholesterol.

BUDGET PLAN:

a. Salaries

Principal Investigator	10%	$ 2,000.00
Biochemist III	50%	5,190.00
Medical Laboratory Technician II	100%	5,389.00
Secretary (Clerk-Steno III)	50%	2,694.00
Fellow in Cardiology	100%	6,000.00
Pension (5.7%)		1,212.56
Workmen's Compensation ($.31 per $100)		66.03
Social Security		893.46
		$ 23,445.05

b. Consumable Supplies

Dogs 40 @ $15.	600.00
Vaccines and Medicine @$1.50 each	60.00
Maintenance @ $.50 per dog per day	2,500.00
Rats 200 @$1.25	250.00
Maintenance for 16 weeks	320.00
Glassware	500.00
Chemicals	1,200.00
	$ 5,430.00

c. Permanent Equipment

NONE

d. Overhead (15% of a and b) $ 4,331.26

GRAND TOTAL $ 33,206.31

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8. FACILITIES AND STAFF AVAILABLE:

(1) General Facilities--

a. A 2,500 bed municipally owned and operated general hospital staffed by the faculties of 5 medical schools, both full-time and part-time. A large percentage of the beds are used for cardiovascular cases. Specialty clinics which are staffed and used by the Division of Cardiology include an adult and pediatric heart clinic, hypertension clinic, peripheral vascular clinic and diabetic clinic among others. The Division of Cardiology is responsible for all problems relating to the subject of cardiovascular disease for the entire hospital.

b. The Division of Cardiology Consists of:

1. A Heart Station equipped with complete instrumentation for cardiovascular diagnosis: Electrocardiography, Radioelectrocardiography, Phonocardiography, Vectorcardiography, Ballistocardiography, Fluoroscopy (Image Intensifier), Cardiac Catheterization, Angiography, Peripheral Vascular Diagnosis (Constant Temperature Room), a Library and a Reading Room.

2. A Biochemical Laboratory devoted fully to cardiovascular research (distinct from the hospital clinical laboratory). It is equipped for doing lipid analysis, catecholamine analysis, blood gas analysis, flame photometry, electrophoresis, osmometry, spectrophotometry, colorimetry, chromatography, polarography, etc.

3. An Isotope Laboratory for radioactive tracer studies

4. An Animal Laboratory

5. An Enzyme and Drug Evaluation Laboratory

6. A Pulmonary Function Section

(2) Major Items of Permanent Equipment (Biochemical Laboratory):

a. Well-type scintillation counter, scaler, probe, rate meter, and pulse height analyzer (NRD).
b. A vibrating reed electrometer (Nuclear-Chicago) with slide chamber, glassware, ionization chambers for measuring weak Beta radioactivity of Tritium, Carbon-14, and Sulphur-35, in solid, liquid and gaseous phase.
c. Ultracentrifuge
d. Refrigerator centrifuge
e. Fraction collector
f. Column chromatography equipment
g. Gas chromatography equipment
h. Paper electrophoresis equipment
i. Densitometer (Photovolt)
j. Spectrophotometer (Beckman)
k. Fluorimeter (Farrand)
l. Densitometer (Photovolt)

m. Colorimeters (Evelyn-Klett)
n. Osmometer (Fiske)
o. Van Slyke volumetric & manometric apparatus
p. Scholander respired air analyzer
q. pH meters (Beckman Model G & Metrohm)
r. Polarograph
s. Respirometer
t. Dual count rate meter, recorder and scintillation counter combination.

3. Staff Available:

Director of the Division of Cardiology
Section Chief: Section of General Biochemistry
Section of Radioisotope Studies
Section of Adult Hemodynamics.
Section of Pediatric Hemodynamics
Section of Peripheral Vascular Disease
Section of Drug Biochemical Laboratory
Section of Animal Research
Clinical Section
Electronics Section

5 Attending Physicians
1 Electronic Engineer
10 Residents & Fellows in Cardiology
2 Technicians
5 Laboratory Technicians

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3. Bellet, S.; Kershbaum, A.; Khorsandian, R.; Caplan, R.F. and Feinberg, L.J.; The Role of Catech olamines in the Free Fatty Acid Response to Cigarette Smoking; Circulation, 28:52, 1963.

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